TRIAL OF A QUANTIFIED MASS REARING METHOD FOR *TOXORHYNCHITES SPLENDENS*[1]

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In recent years there has been considerable interest in laboratory rearing of *Toxorhynchites* mosquitoes for use as biological control agents (Vongtangswad and Trpis 1980) and for the assay of dengue viruses (Eshita et al. 1982). Although larvae of these mosquitoes are cannibalistic (Steffan and Evenhuis 1981), mass rearing methods have been described for some species which have yielded pupation rates from 73 to 94%, provided adequate prey density is maintained (Focks et al. 1977, Focks and Boston 1979, Horio and Tsukamoto 1985).

In our laboratory, *Tx. splendens* (Wied.) are reared for dengue virus isolation. In order to increase production efficiency and reduce inputs of time and manpower, we attempted to employ mass rearing methods similar to those described by Focks and Boston (1979).

Five hundred 0 to 24-hour-old *Tx. splendens* eggs were counted and placed in a 54 × 45 × 6.75 cm plastic developing tray containing 5 liters of water. Approximately 10,000 newly hatched *Aedes aegypti* larvae and 100 ml of slurry (Focks and Boston 1979) were added to the tray.

A second tray was set up containing 4 liters of water, ca. 6,500 *Aedes aegypti* (Linn.) eggs, and 70 ml of slurry. The larvae from this tray were added to the first tray 3 days later. A fresh tray was set at each feeding and was added to the predator tray when *Ae. aegypti* larvae were 4 days old. Thus, additional prey were added on days 4, 7, 10 and 13.

Prey larvae were deliberately underfed to retard pupation. In preliminary trials of this method when prey larvae were provided with greater amounts of food [i.e., a second feeding as described by Focks and Boston (1979)], pupation and emergence of adult *Ae. aegypti* from predator pans became a problem as all prey larvae were not consumed between feedings.

Trays were examined daily. Live pupae, dead pupae, and dead larvae were counted and removed. Pupae were rinsed and placed in "Mosquito Breeders" (BioQuip Products)[2] in 250 ml of fresh water at a maximum density of 50 pupae per container until adult emergence.

The insectary was maintained at a temperature of 24 to 29°C and 50 to 85% RH. One hundred and seventy-six trays of *Tx. splendens* were reared in this manner between June and November, 1986.

The egg viability rate, as monitored in weekly samples of 50 eggs hatched individually, was 62.3 ± 10.1%. This would indicate that an average of 311.5 larvae hatched from each set of 500 eggs. Focks and Boston (1979) do not report a hatching rate in their trials, but since they recovered 245 pupae from a set of 330 eggs, initial larval densities were probably similar. An average of 60.8 ± 19.1 pupae were recovered from each tray. Larval mortality was then an estimated 80.5%, or 250.7 larvae per pan. The average length of the larval period was 16.8 ± 1.4 days. Average length of the pupal period was 5.5 ± 0.3 days. The estimated mean pupation rate in the trays was only 19.5% (60.8 pupae per 311.5 larvae). This was far below rates reported by Focks and Boston (1979) for *Tx. rutilus rutilus* (Coq.) and Horio and Tsukamoto (1985) for *Tx. towadensis* Matsumura.

Presumably, most larval mortality was due to cannibalism. As Focks and Boston (1979) observed, the great majority of this occurred early in development. Only 6.6% of larval mortality was observable in the form of a whole or partial cadaver in the tray. Once larvae reached the third instar, there was relatively little noticeable reduction in their numbers.

Pupal mortality was very high at 44.3%. Thus, the overall yield of the system from egg to adult was only 6.8%. Pupal mortality was not due to crowding in the emergence container. Over a period of 14 days, mortality was observed in 2 groups of pupae. Each day half the pupae produced were placed singly in small cups until death or adult emergence, while the other half

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[2] Mention of a commercial product does not constitute a recommendation of its use by the Navy Department.

was placed in emergence containers in the standard manner. There was no difference in mortality between the 2 groups. Fifty-two percent (183/349) of pupae placed individually in cups and 51.3% (181/353) of the pupae in emergence containers died (Chi square = 0.0913, not significant). In our laboratory, *Tx. splendens* is also reared individually and pupae are placed in groups in emergence containers in the same manner as mass reared pupae. Pupal mortality has not been a problem among individually reared pupae.

Aeromonas hydrophylla was cultured from dead pupae and was the suspected cause of pupal mortality. This hypothesis has not yet been tested experimentally.

Due to the high rates of larval and pupal mortality, this method of rearing was discontinued. The reason for the low yield of *T. splendens* with these methods is not clear. Prey densities were maintained above levels at which it has been stated that cannibalism rarely occurs in *T. rutilus septentrionalis* (Dyar and Knab) (Trimble and Corbet 1975). At no time did *Tx. splendens* larvae consume all the prey larvae provided and surplus prey were present in the trays at each feeding. This particularly became a problem at later feedings when *Tx. splendens* density was low and *Ae. aegypti* pupae had to be removed from predator pans to prevent adult emergence in the insectary.

Eshita et al. (1982) also reported relatively low yields of *Tx. amboinensis* (Doleschall), a species closely related to *Tx. splendens* (Steffan and Evenhuis 1985), when using a mass rearing method. They obtained a pupation rate of only 29.9%.

A genetic basis for cannibalistic behavior has been suggested. It has been shown that cannibalistic tendencies may differ markedly between species (Fox 1975). It is possible that *Tx. splendens* is more aggressive towards its conspecifics than other members of the genus. Muspratt (1951) cites earlier authors who noted the strong cannibalistic tendencies of this species.

We believe this is the most likely explanation for the low yields we obtained. Focks and Boston (1979) reported combining remaining *Tx. rutilus rutilus* larvae into trays containing about 150 larvae following a single pupal "picking" on day 15. When we tried this technique with fourth instar *Tx. splendens*, intense intraspecific killing behavior severely reduced the number of larvae per tray, further evidence of behavioral differences between the two species. It is also possible that our laboratory colony represents a particularly cannibalistic strain of this species.

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